

Neutron scattering from magnetically aligned biomimetic substrates

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Abstract. Binary mixtures of long-chain and short-chain phospholipids or bile-salt analogues can form disk-shaped bilayered micelles (diameter 10–100 nm, thickness 4–6 nm) which have been shown to orient themselves in a magnetic field, serving as a self-orienting biomimetic substrate. Within the magnetic field the bicellar system doped with lanthanide ions is highly alignable ($< 1.0^\circ$ C mosaic spread FWHM) and shows a temperature- and concentration-dependent nematic-to-smectic phase transition. The structural and thermodynamic changes during this phase transition at different hydration levels were investigated by small-angle neutron scattering and differential scanning calorimetry. While the neutron scattering data indicate a singular phase transition, the calorimetric measurements show a rather complex phase behavior of the system between 283 and 303 K.

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Lipid mixtures composed of short- and long-chain phosphatidylcholines such as dihexanoyl phosphatidylcholine (DHPC) and dimyristoyl phosphatidylcholine (DMPC), are extensively studied [1–6] as a potential biomimetic “substrate” capable of aligning a variety of macromolecules. These magnetically oriented bilayered micelles or commonly named “bicelles” are believed to achieve diameters of between 10 and 100 nm, depending on the molar ratio of DMPC to DHPC [1]. The bicelles are known to align with their bilayer normal, either perpendicular to the applied magnetic field B (negative ordering), or parallel to B (positive ordering), depending on the magnetic susceptibility of the bilayer molecules [7–9].

Doping the bicelle system with paramagnetic lanthanide ions (i.e., Eu^{3+} , Er^{3+} , Tm^{3+} or Yb^{3+}) [1, 3, 4, 6] change the bilayer’s susceptibility adopting parallel ordering ($n \parallel B$). In addition doping of the bicelle system with Tm^{3+} improved the alignment of the bilayered mixtures ($< 1.0^\circ$ C mosaic spread FWHM) and transformed the system from a discotic

nematic phase into a lamellar smectic phase [4, 6]. Interlamellar repeat spacing of the aligned bicelles evolve up to several hundred Ångström as a function of water content [10].

At lower temperatures (< 298 K), the mixture has a low viscosity and can not be aligned in a magnetic field. At higher temperatures the liquid crystal phase of a DMPC/DHPC mixture is gel-like and has an intrinsic negative ordering ($n \perp B$) at molar ratios of DHPC: DMPC in the range of 1:2–1:6, water content of 97–60% w/v [11]. Increasing the temperature in the aligned DMPC/DHPC system transforms the bicellar system into a perforated lamellar phase [10].

Using small angle neutron scattering (SANS) and differential scanning calorimetry (DSC) we have investigated the phase behavior in the DMPC/DHPC mixture at various water to lipid ratios.

1 Experimental

Phospholipids di-hexanoyl-phosphatidylcholine (DHPC) and di-myristoyl-phosphatidylcholine (DMPC) were purchased from Avanti Polar Lipids (Alabaster, AL). Samples were prepared in D_2O as described [4]. Neutron scattering experiments were performed at the SANS instrument V4 at BENSC, HMI, Berlin, Germany. Magnetic fields up to 4.5 T were applied using the vertical cryomagnet VM3 at BENSC. Differential scanning calorimetry was performed with a MC-2 microcalorimeter (MicroCal, Northampton, MA) without applying magnetic field.

2 Results and discussion

In the low viscosity phase below 298 K an isotropic scattering pattern of the randomly oriented bicelle system is observed at applied magnetic field (see Fig. 1). A highly anisotropic scattering pattern of the smectic phase evolves increasing the temperature. The pattern is consistent with a smectic phase transformed into a well aligned lamellar arrangement with the bilayer normal parallel to the applied magnetic field [10, 12]. Interlamellar repeat spacing of the

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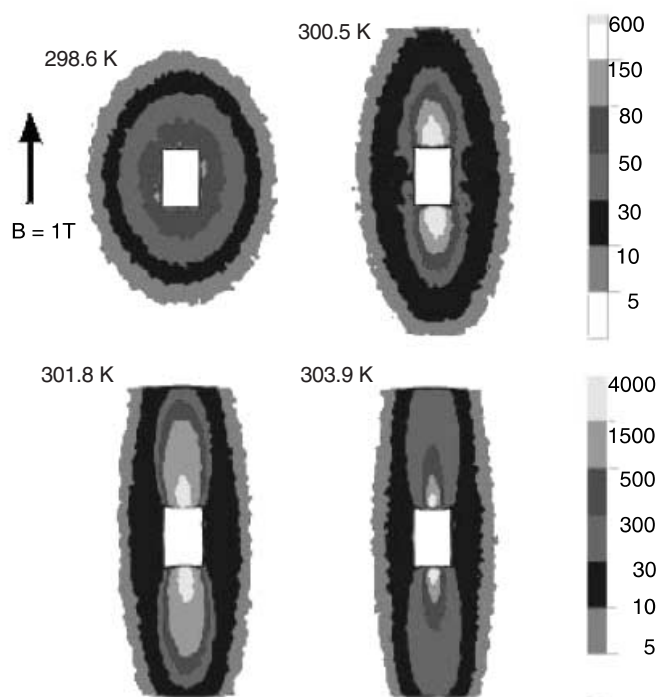


Fig. 1. 2D-SANS pattern for DMPC/DHPC (3.2:1 molar ratio) bicelles (92 wt% D₂O, DMPC/Tm³⁺ molar ratio 6:1) during nematic-to-smectic phase transition. $B = 1$ T

aligned lamellae increase with increasing water content from 6.0 nm at 77 wt%, 21.3 nm at 88 wt% and 28.8 nm at 92 wt% D₂O. These values were independent of the applied magnetic field.

While the neutron scattering data indicated a singular structural phase transition as described also with solid state NMR [11] first calorimetric measurements showed a more complex phase behavior of the system between 283–303 K. The obtained DSC scans for the mixtures investigated, 77, 88 and 92 wt% D₂O can be simulated with four different single transitions (Fig. 2). Magnetic field was not present in these measurements. For 92 wt% D₂O starting at 287.9 K a first transition occurs, followed by transitions at 290.8 K, 293.7 K and 297.7 K. The first and last value are close to the pre- and main-transition of pure DMPC, slightly shifted to higher values. The additional two transition temperatures in between seem to be unique to the observed bicellar systems. The origin of these transitions are unknown and were not observed in other work [10, 11]. It can only be speculated if these sub-

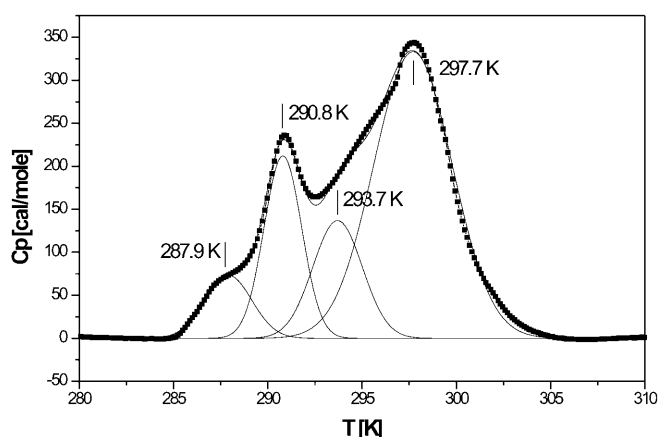


Fig. 2. DSC up-scan of DMPC/DHPC (4.4:1 molar ratio) bicelles (92 wt% D₂O) during nematic-to-smectic phase transition. $B = 0$ T

transitions could indicate pre-alignment and/or re-orientation states of the bicelles due to partial melting and mixing of the long- and shortchain phospholipids mixed. Nevertheless they support the recent description of co-existence of perforated lamellae or lamellar sheets with aligned bicelles [10, 12]

The present calorimetric data indicate, that the dramatic change in the scattering pattern clearly reflects a distinct thermodynamic transition of the aligned DMPC/DHPC bicelles which would not occur if only an alignment process of a nematic-to-smectic phase transition would be present.

References

1. R.R. Vold, R.S. Prosser: *J. Magn. Reson. B* **113**, 267 (1996)
2. C.R. Sanders, B.J. Hare, K.P. Howard, J.H. Prestegard: *Prog. NMR Spectro.* **26**, 421 (1994)
3. R.S. Prosser, S.A. Hunt, J.A. DeNatale, R.R. Vold: *J. Am. Chem. Soc.* **118**, 269 (1996)
4. J. Katsaras, R.L. Donaberger, I.P. Swainson, D.C. Tennant, Z. Tun, R.R. Vold, R.S. Prosser: *Phys. Rev. Lett.* **78**, 899 (1997)
5. J. Katsaras, *Physica B* **241**, 1178 (1998)
6. R.S. Prosser, J.S. Hwang, R.R. Vold: *Biophys. J.* **74**, 2405 (1998)
7. K.D. Lawson, T.J. Flautt: *J. Am. Chem. Soc.* **89**, 5489 (1967)
8. B.J. Forrest, L.W. Reeves: *Chemical Reviews* **81**, 1 (1981)
9. J. Seelig, F. Borle, T.A. Cross: *Biochim. Biophys. Acta* **814**, 195 (1985)
10. M.-P. Nieh, C.J. Glinka, S. Krueger, R.S. Prosser, J. Katsaras: *Langmuir* **17**, 2629 (2001)
11. G. Raffard, S. Steinbruckner, A. Arnold, J.H. Davis, E.J. Dufourc: *Langmuir* **16**, 7655 (2000)
12. M.-P. Nieh, C.J. Glinka, S. Krueger, R.S. Prosser, J. Katsaras: *Biophys. J.* **82**, 2487 (2002)